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EXTRACORPOREAL PERFUSION WITHOUT EXOGENOUS ANTICOAGULATION:

ITS PROTECTIVE ROLE IN ENDOTOXIN SHOCK

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ABSTRACT

$\longrightarrow$ Our previous work demonstrated that after 90 minutes of arteriovenous
perfusion without anticoagulation dogs were protected from lethal endotoxin.
The present study extended that work by conducting experiments with heparinized
and nonheparinized, perfused and nonperfused animals administered endotoxin.
Experiments with 42 adult mongrel dogs were divided into six groups: Group A
(endotoxin; no perfusion); B (perfusion; no endotoxin); C (perfusion; endotoxin
initiated after 90 minutes); D (perfusion and endotoxin simultaneously); E
(endotoxin plus heparin; no perfusion); F (perfusion plus heparin; endotoxin
initiated after 90 minutes). Percent permanent survival (> 7 day): Group A =
14%; Group B = 100%; Group C = 86%; Group D = 29%; Group E = 29%; Group F = 57%.
Survival results from Group A document the degree of lethality of the endotoxin.
Observations from Group B confirm the physiological stability of the perfusion
preparation. Findings from Group C document that 90 minutes preperfusion
provides excellent defense against the lethality of endotoxin shock. Whole
blood clotting time (WBCT) decreased in nonperfused dogs (Group A), following
endotoxin while perfusion alone (Group B) and perfusion followed by endotoxin
(Group C) were associated with increases in WBCT and numbers of immature neu-
trophils. Arterial pressure and blood glucose, total white blood cell, plate-
let, fibrinogen, and factor V concentrations were unaffected by perfusion but
fell sharply in all groups after endotoxin. The decrease in numbers of neutrophils
following endotoxin was much less, and the increase in immature   ophils
was significantly greater, in the preperfused animals which had received endotoxin
(Group C) than in the animals that received endotoxin alone (Group A). Results
underscore the following: (a) Arteriovenous extracorporeal pump perfusion
without exogenous anticoagulation is not harmful: All animals were permanent
(> 7 day) survivors. (b) Death from endotoxin is prevented by preperfusion of
90 minutes but not when endotoxin is administered at the onset of perfusion.
(c) Intravenous heparin administration offers no discernible protection against
endotoxin shock in either perfused or nonperfused animals. (d) Increased

numbers of circulating immature neutrophils are associated with protection
from the lethal effects of endotoxin. This may be an important marker of
the adequacy of host response to endotoxin. (e) Increasing venous return
to the heart by an arteriovenous perfusion system is not a significant
mechanism responsible for protecting animals administered endotoxin.

Key words: white blood cell changes in shock, survival from endotoxin shock,
endotoxin shock, therapy of shock, perfusion during shock, coagulation changes
in endotoxin shock

Our previous work demonstrated that after 90 minutes of extracorporeal arteriovenous perfusion without exogenously administered anticoagulants dogs were protected from lethal endotoxin administration (1). In those experiments 2 mg/kg E. coli endotoxin was infused for 30 minutes after whole blood clotting times had reached maximal values in six dogs. All six perfused dogs survived and appeared healthy one week later when sacrificed. Five of six nonperfused dogs given the same dose of endotoxin died. Several factors were thought to account for the increased survival rate: augmentation of venous return, preservation of metabolic status, stimulation of host defense, and prevention of disseminated intravascular coagulation (1-6). The purpose of this study was to assess the mechanisms accounting for the protection of dogs against endotoxin shock as previously reported (1). Findings suggest that stimulation of the host defense system plays a major role in the protection with negligible ancillary roles for augmented venous return and anticoagulation factors.

MATERIALS AND METHODS

Forty-two adult mongrel dogs of either sex weighing 18.5 ± 0.3 kg with hematocrits of at least 37% and white blood cell counts less than $22,000/mm^3$ were divided into six groups (N=7/group). All dogs were tested for microfilaria (heartworms) and were negative. Prior to experimentation, each dog was fasted 18 hours but given water ad libitum. The morning of the experiment, each was anesthetized with sodium pentobarbital 25 mg/kg body weight. The study consisted of two groups of nonperfused dogs and four groups perfused with a modification (open air reservoir removed) of our previously described extracorporeal perfusion system (1, 7, 8). Five of six groups were given E. coli endotoxin (lipopolysaccharide B:055:B5; Difco Laboratories, Detroit). Two groups were systemically heparinized before and during experimentation. The following is a brief description of the six groups:

Group A: Endotoxin administered only; no perfusion. This group served to establish the lethality of the endotoxin. *Group B: Perfusion only; no endotoxin given.* This group served to demonstrate the physiological stability of the perfusion system. *Group C: 90 minutes of perfusion; then endotoxin administered.* This group served to substantiate the ability of our extracorporeal perfusion system without exogenous anticoagulation to protect against endotoxin. *Group D: Endotoxin infusion initiated at the onset of perfusion.* This group served to ascertain whether simultaneous perfusion would be as effective as prior perfusion against the lethal effects of endotoxin. *Group E: Heparinized dogs given endotoxin; no perfusion.* This group served to evaluate the effects of heparin on recovery from endotoxin shock. *Group F: Heparinized dogs perfused 90 minutes; then given endotoxin.* This group served to assay the effects of exogenous heparin plus perfusion on recovery from endotoxin shock.

Perfused dogs. Before surgical incisions were made, the right brachial and both femoral areas of each dog were shaved and scrubbed with betadine. The brachial artery was then cannulated to provide access for recording hemodynamic parameters and sampling blood. One femoral vein was cannulated and connected to a Harvard Apparatus Infusion Pump for use when endotoxin was administered (Groups C, D, and F). Both femoral arteries and one femoral vein were surgically isolated and tagged with silk sutures in preparation for perfusion.

The extracorporeal circuit consisted of Y-shaped large bore flexible plastic (Tygon®) tubing with the ends tapered to facilitate their insertion into the femoral arteries and vein. The tubing was completely filled with isotonic saline and clamped with hemostats to prevent saline loss and blood reflux during cannulation. Perfusion of Groups B, C, and D was accomplished without the use of any exogenous anticoagulant. Our previous perfusion system was open to the air (1, 7, 8). We closed the system in this study by removing

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the reservoir. Two ends of the saline filled tubing were advanced into the distal aorta of each dog via the left and right femoral arteries. The other end of the tubing was threaded through a nonocclusive roller-type pump (Medical Specialty Co., Forth Worth, Texas) and inserted into a femoral vein. To initiate perfusion, clamps were simultaneously released, the pump started and set at approximately 900 ml/min. Following four hours of continuous perfusion, the blood in the extracorporeal tubing was returned to each dog and the blood vessels were ligated and cannulas removed.

Nonperfused dogs. The brachial artery and one femoral vein of the non-perfused dogs (Group A and E) were surgically cannulated like those of the perfused dogs described above.

Endotoxin-shocked dogs. In the experiments evaluating endotoxin (all groups except B), 2 mg/kg E. coli endotoxin was dissolved in sterile saline and infused over 30 minutes via the femoral vein.

Nonheparinized and heparinized dogs. No animal in Group A, B, C, or D received an exogenous anticoagulant of any kind. All catheters and pressure transducers were maintained with nonheparinized saline.

Dogs in Groups E and F were systemically heparinized throughout the experiments. Each was given an initial bolus of 150 U heparin/kg then maintained with an infusion of approximately 100 U/kg/hr (9). Heparin blood levels were assayed throughout the experiment and were adjusted to achieve approximately 1 U/ml blood.

At the end of the 4 to 6 hour experiment, and twice daily for four days, each dog was given gentamicin sulfate intramuscularly, 4.5 mg/kg. Animals alive and healthy seven days after the E. coli insult were considered "survivors". During the acute experiment, rectal temperature was maintained above 37°C with heating pads, and 6% dextran was administered to maintain hematocrit at control level. Baseline parameters and blood samples were taken just prior to initiation of perfusion or endotoxin (whichever came first). Parameters

measured periodically during the 5 hour period included mean systemic arterial pressure (MSAP), heart rate, hematocrit, rectal temperature, pH, blood glucose, platelet and white blood cell concentrations, Lee-White whole blood clotting time, fibrinogen, factor V, heparin, and partial thromboplastin time (PTT). These parameters were measured as previously described (1, 7, 8).

Data were analyzed using the Student's t test for paired and unpaired data and the Fisher's exact test for survival statistics. Only values $p \leq 0.05$ were considered statistically significant.

RESULTS

Is the perfusion preparation a stable model and is preperfusion neces-sary to provide protection against the lethal effects of endotoxin?

The first series of experiments (Group B) were conducted to determine the responses of animals to extracorporeal perfusion without exogenous anticoagulation. Endotoxin was not administered. Table I summarizes the findings from seven dogs. Mean systemic arterial pressure, blood glucose and partial thromboplastin time were relatively unchanged, while mild alterations in platelets and fibrinogen occurred during the four hour per-fusion period. Of special significance were the progressive increases ($p < 0.05$) in whole blood clotting time and white blood cell concentrations: clotting times rose progressively from a mean control value of 7.1 minutes to 11.3 minutes during the four hour period. White blood cell concentrations increased from 10.5 x 10^3/mm^3 to 18.1 x 10^3/mm^3 during the same period. All animals from Group B tolerated the perfusion well, recovered from the anesthesia and surgical incisions uneventfully and were permanent survivors (> 7 day). (For survival data, see Table II).

The second series of experiments (Groups A and C) was conducted to substantiate our prior work (1) documenting the ability of extracorporeal perfusion without exogenous anticoagulation to protect against endotoxin *and* to determine whether simultaneous perfusion would also protect against endo-toxin lethality (Groups A, C, D). Table II summarizes the survival results. A significant difference was seen between animals receiving endotoxin but not perfused (Group A; 1 of 7 surviving) and animals perfused for 90 minutes followed by endotoxin (Group C; 6 of 7 surviving, $p = 0.01$). In contrast, dogs given endotoxin at the onset of perfusion (Group D) were not protected against endotoxin (2 of 7 surviving). There was a statistically significant

increase in survival in animals perfused for 90 minutes (Group C) and then given endotoxin, in contrast to those given endotoxin at the onset of perfusion (Group D; $p = 0.05$). There was no difference in survival ($p > 0.05$) between animals perfused without endotoxin (Group B) and those receiving endotoxin following 90 minutes of perfusion (Group C).

With regard to the cardiovascular measurements of Groups A, C, and D (Figure 1): (a) Heart rates were variable. (b) Similar decreases in mean systemic arterial pressure were seen in the three groups (Figure 1). It is noted, therefore, that despite the vast differences in rates of survival, blood pressure changes were not of prognostic value for survival.

Figure 2 illustrates changes in blood glucose and whole blood clotting time (WBCT) for Groups A, C, and D. WBCT had significantly increased in Group C before endotoxin was given, progressively increased following endotoxin, and then fell following cessation of perfusion. WBCT in nonperfused dogs decreased significantly 60 minutes after endotoxin administration, but otherwise was relatively stable.

Why is 90 minutes of perfusion prior to endotoxin administration protective?

Table I showing the host defense response of Group B provided a clue: perfusion without endotoxin elicited a significant rise in WBC concentrations by 3 hours. Findings from Group C (Figure 3) demonstrated that WBC concentrations of preperfused animals fully recovered to control values by 210 minutes post endotoxin. In contrast, the total WBC concentrations of Groups A and D all remained significantly depressed at 210 minutes after endotoxin administration. Figure 3 also shows that Groups A and D differed markedly from C in survival. Differential analysis of the total WBC concentrations of the three groups shows that the more rapid recovery of WBC concentrations in Group C was due primarily to increases ($p < 0.05$) in numbers of circulating immature neutrophils.

Given the rapid recovery of total WBC concentrations seen in Group C
(Figure 3), we reexamined the WBC response during the 90 minute perfusion
(prior to endotoxin administration). We discovered that while there were
no significant increases in total WBC concentrations until after the 90
minute interval, there was a significant increase (p < 0.05) in absolute
numbers of immature neutrophils within the 90 minute interval. Furthermore,
following endotoxin, six of the seven animals in Group C reached an *absolute*
number of 1000 or more immature neutrophils by 180-240 minutes post-endotoxin,
and only two of the nonperfused animals in Group A reached absolute numbers
of 1000. This difference is even more evident if one compares the *percent*
immature neutrophils seen after 180-240 minutes. All of the perfused animals
exceeded 20% immature neutrophils while all but one of the nonperfused
animals failed to reach 20% of this cell population by 180-240 minutes.

Another way of examining the relevance of the above associations is
to correlate survival with the percentage of circulating immature cells in
all six groups of dogs given endotoxin. We compared the number of immature
cells observed in the 15-60 minute interval following onset of endotoxin
infusions. Table III shows that if 15% is used as a "cut off" to arbitrarily
establish two populations, twelve animals with greater than 15% immature
neutrophils lived and only one died, whereas eighteen animals with less than
15% died and only three lived (p < 0.001).

How does extracorporeal perfusion stimulate the animal to increase the
number of circulating immature neutrophils?

Previous observations have shown that cortisol elaboration stimulates
WBC mobilization and release (10, 11). Therefore, we conducted separate
experiments to determine the relationship of endogenous cortisol and WBC
concentrations of animals when placed on extracorporeal perfusion without
exogenous anticoagulation. The study consisted of eight dogs perfused as
previously described (8). Serum cortisol levels were measured by radioimmun-

oassay according to the method of Kling and Westphal (12) during a three
hour perfusion period and following the termination of perfusion for three
hours. Endotoxin was not administered. Table IV shows that after a prolong-
ed increase in cortisol concentration sustained since 15 minutes after the
onset of perfusion a rise in WBC concentration above control values occurred
at 240 minutes. Both mature and immature cells were involved thus impli-
cating mobilization of mature WBC's from marginated sites and release of
immature cells from the bone marrow as a result of the extracorporeal perfusion.
Changes in mean systemic arterial pressure, heart rate and blood glucose
concentrations were relatively insignificant.

*What role is there, if any, for anticoagulation in the protection of
animals from the effects of endotoxin?*

The third series of studies was conducted (Groups E and F) to deter-
mine if exogenous anticoagulation would give the animals an edge. Would
preventing disseminated intravascular coagulation (DIC) with heparin improve
the cardiovascular status and increase the survival rate? Findings demon-
strated, however, that heparin administration did not protect dogs against
the lethal effects of endotoxin; survival rates were not improved with heparin
($p > 0.05$) in either perfused or nonperfused animals (Table II). The results
shown in Figure 4 compare the mean systemic arterial pressures between endo-
toxin-shocked animals perfused and nonperfused with and without exogenously
administered heparin. Animals perfused with exogenous heparin had significantly
lower arterial pressures ($p < 0.05$) during most of the perfusion period.
Nonperfused animals given heparin demonstrated lower average pressure values
during the post-endotoxin period. Figure 5 shows that heparin administration
also did not modify the recovery rate of the white blood cell or platelet
concentrations of the preperfused animals given endotoxin.

With respect to the response of coagulation factors to endotoxin, Figure 6
shows that platelets, factor V, and fibrinogen fell abruptly in animals that

received endotoxin alone (Group A). The same was true in animals that were preperfused without heparin (Group C) and preperfused with heparin (Group F). However, in spite of similarities in coagulation responses in the three groups survival rates (Table III) in Groups A and F are 1/7 and 4/7 respectively while that in Group C is 6/7.

DISCUSSION

The primary purpose of the present study was to examine the mechanism of protection of extracorporeal perfusion without exogenous anticoagulation against the lethal action of endotoxin in dogs. The perfusion system itself was also evaluated for its effects on animals. Results from the present study corroborate our previous work (1, 7, 8) in that the perfusion system per se has no adverse effects on the dog and provides remarkable protection against endotoxin shock if ninety minutes of perfusion is carried out prior to administration of endotoxin.

A finding of chief importance providing a possible explanation for the observed protection of the extracorporeal system against the lethal action of endotoxin was that increased numbers of immature neutrophils correlate with increased survival of dogs given endotoxin. Preperfusion of animals with the extracorporeal system without exogenous anticoagulation protected the animals from the lethal effects of endotoxin. The present study showed that one of the effects of perfusion is an elevation in the concentration of circulating immature neutrophils. A high correlation was observed between the increase of immature neutrophils and survival from lethal endotoxin. While no direct evidence is presented that immature neutrophils are a part of the mechanism by which preperfusion protects, they clearly are reliable markers or predictors of whether or not the animal will survive.

There are many published reports supporting the view that neutrophils (polymorphonuclear leukocytes) perform a key role in the defense against E. coli or endotoxin-induced shock or human septic shock. Neutrophils have been designated the body's first line of defense by Stossel (13). Cline et al (14) and Cohn and Morse (15) report that endotoxin is efficiently phago-cytized by neutrophils. Although the intimate interaction of endotoxin and neutrophils is not understood (16), neutrophils localized in the vasculature of the lung, spleen, liver and kidney as well as those circulating systemically

take up and or bind endotoxin and result in its detoxification and inactivation (16, 17).

The observation that cortisol concentrations rise during extracorporeal perfusion with our system and that these increases are associated with elevations in neutrophil concentrations, provides an explanation for the protection of our perfusion system against the lethal action of endotoxin. Leukocytosis is also known to occur when glucocorticosteroids are administered exogenously (10,11). Dale and Fauci and others (10,11) point out that the mechanism of the increase in leukocyte blood concentration after steroid administration is the release of neutrophils from the bone marrow and the simultaneous reduction of their passage from the blood to other tissues which effectively lengthens their life-span in blood (11). Chemotactic, phagocytic, and bactericidal properties of the newly released neutrophils are reported to be normal in all respects (18-20), and they should therefore substantially augment the host defense system. Studies by Archer and others(21) support the view that increased numbers of neutrophils protect liver function and enhance survival in canine endotoxin shock. An increase in the neutrophil's basal metabolic activity, as well as increased total numbers of neutrophils should be primary factors in enhancing the efficiency of the host defense system and increasing the survival rate of animals given endotoxin (21-23).

Recent reports have described the beneficial effects of transfused white blood cells as treatment for experimental bacteremia in dogs (24,25). Graw and others (26) have reported a significant increase in the survival rate of neutropenic patients in gram-negative sepsis when they are transfused with white blood cells.

The important function of the neutrophil as a significant factor in the defense against endotoxin or septic shock appears to be clearly established (10, 11,13-26). Results from the present study further strengthen this conclusion by demonstrating a correlation between the concentration of neutrophils in circulating blood and the survival rate of dogs administered endotoxin.

A second feature of note in the present study was the increase in whole blood clotting time (WBCT) observed during perfusion without exogenous anticoagulation as contrasted with an average decrease in clotting time in nonperfused animals administered endotoxin. Is there a relationship between WBCT and survival? This is uncertain. What is certain is that regardless of the WBCT response, there is a *consumption* of clotting factors in *both* the surviving and nonsurviving dogs (perfused and nonperfused) and in dogs which were given heparin.

Thus, administration of heparin did not protect dogs from the lethal effects of endotoxin, nor did the late response or consumption of coagulation factors following endotoxin serve as a marker or predictor of whether or not an animal would survive. Furthermore, since these factors were consumed in both preperfused animals which do survive and in the nonperfused animals which die, it seems unlikely that the late consumptive (DIC) events are causally associated with the lethal effects of endotoxin. This seems particularly true in light of the long interval between endotoxin infusion and consumption of fibrinogen and factor V. Thus, there is no apparent association between late responses of coagulation factors, disseminated intravascular coagulation, and survival. However, the early nonconsumptive response of the coagulation system as reflected in the WBCT in the preperfusion studies cannot be ruled out as playing a role in the protection offered by preperfusion against endotoxin.

Finally, survival rates were statistically similar whether heparin was administered or not to nonperfused or perfused animals given endotoxin. Mean arterial blood pressures were generally lower in the heparinized animals (Groups E, and F), but this was not reflected in survival rates. The problem of lowered systemic arterial pressure during and following extracorporeal perfusion (27-31) might be due to internal bleeding and subsequent reduction in circulating blood volume caused by heparin therapy.

Most of the parameters measured in the present study were of no use in predicting the eventual outcome of endotoxin shock: essentially no significant differences in arterial pressure, heart rate, blood glucose and platelet concentrations were seen in perfused compared with nonperfused animals. Augmentation of venous return reported to be of benefit to dogs administered endotoxin (5,6) proved to be insignificant in terms of ultimate survival. This was demonstrated by infusing endotoxin while simultaneously perfusing animals (Group D; 2/7). Measured changes in coagulation factors did not aid in explaining the protective nature of extracorporeal perfusion. In fact, the late consumption of coagulation factors following endotoxin did not even serve as a marker or predictor of whether an animal would survive. Since these factors were consumed in both preperfused animals which survive and in nonperfused animals which die, it seems unlikely that late consumptive events are causally associated with the lethal action of endotoxin. Early subtle alterations in the coagulation factors may have occurred during the ninty minute perfusion period which presented more suitable conditions for host defense and peripheral perfusion when endotoxin was infused, but these have not been identified.

ACKNOWLEDGEMENTS

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TABLE I

RESPONSE OF DOGS TO EXTRACORPOREAL PERFUSION

WITHOUT EXOGENOUS ANTICOAGULATION

(Group B; N=7) (no endotoxin given)

Parameter - Mean (±SE)	Minutes of Perfusion							
	0	15	30	60	90	120	180	240
Mean Systemic Arterial Pressure (mm Hg)	149(5)	152(5)	154(5)	156(5)	155(5)	153(5)	155(5)	153(6)
Heart Rate (/min)	154(8)	181(10)*	181(10)*	181(10)*	183(9)*	179(11)*	176(10)*	176(12)*
Blood Glucose (mg/dl)	91(5)			100(8)		103(8)	101(6)	96(6)*
White Blood Cells ($\times 10^3/mm^3$)	10.5(1.0)			8.7(1.4)		13.7(1.7)	17.4(2.0)*	18.1(2.1)*
Lee White Whole Blood Clotting Time (min)	7.1(0.5)	8.1(0.4)*	8.7(0.5)*	8.9(1.1)	9.3(0.9)	9.9(1.0)*	11.0(1.2)*	11.3(1.2)*
Platelets ($\times 10^3/mm^3$)	285(52)	230(68)		154(43)*		215(35)*	220(30)	200(28)*
Fibrinogen (%)	100(0)	91(4)*	98(4)	88(4)*	85(8)	87(10)	87(7)	81(8)
Partial Thromboplastin Time (sec)	13.7(0.4)	14.9(2.4)	14.1(1.3)	14.8(1.0)	14.4(0.6)	19.0(2.4)	17.3(1.6)	15.1(1.1)

* paired comparison to 0 time ($p = < 0.05$)

TABLE II

EFFECT OF PERFUSION WITH AND WITHOUT EXOGENOUS
ANTICOAGULATION ON SURVIVAL TO ENDOTOXIN

Group (N=7/group)	Number Surviving* Total Number		Percent Survival
A Endotoxin, no perfusion	1/7		14%
B Perfusion, no endotoxin, no anticoagulant	7/7	$p=0.01^{\dagger}$	100%
C Perfusion 90 minutes followed by endotoxin, no anticoagulant	6/7		86%
D Perfusion with endotoxin at onset of perfusion, no anticoagulant	2/7	$p=0.05^{\dagger}$	29%
E Endotoxin, no perfusion, heparin administered prior to endotoxin	2/7		29%
F Perfusion 90 minutes followed by endotoxin, heparin administered prior to perfusion	4/7		57%

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* Survival time = > 7 days

† Each group was compared with every other group using the Fisher Exact Test.
  Values equal to or less than 0.05 are presented.

TABLE III

CORRELATION OF ULTIMATE FATE OF ANIMALS WITH PERCENT IMMATURE NEUTROPHILS CIRCULATING 15 TO 60 MINUTES FOLLOWING ENDOTOXIN INFUSION. GROUPS A, C, D, E, AND F

Number of dogs with less than 15%* immature neutrophils	Number of dogs with more than 15% immature neutrophils
18 Dead	1 Dead
3 Alive	12 Alive

* The survival rates of the two groups significantly differed (p<0.001) using Fisher Exact Test analysis.

TABLE IV

RELATIONSHIP OF SERUM CORTISOL LEVELS TO CIRCULATING WHITE BLOOD CELL LEVELS DURING AND AFTER EXTRACORPOREAL PERFUSION WITHOUT EXOGENOUS ANTICOAGULATION

N=8 (Mean ± SE) (no endotoxin administered)

	HOURS OF PERFUSION					NO PERFUSION			
	0	.25	1	1.5	2	3	4	5	6
Serum Cortisol (ng/ml)	154 ±42	209 ±40 *		206 ±24		235 ±26 *	249 ±34 *	254 ±47 *	198 ±33
Total White Blood Cells (/mm³)	10,300 ±1,600			6,200 ±900 *		9,400 ±1,600 *	15,400 ±2,700 *	19,600 ±3,400 *	23,300 ±3,800 *
Mature Neutrophils (/mm³)	7,400 ±1,300			4,700 ±800 *		8,000 ±1,800	13,700 ±2,900 *	17,800 ±3,500 *	21,400 ±4,000 *
Immature Neutrophils (/mm³)	41 ±23			126 ±42		311 ±127 *	466 ±129 *	811 ±253 *	1,396 ±439 *
Mean Systemic Arterial Pressure (mm Hg)	135 ±4	128 ±7 *	121 ±8 *	119 ±9 *	121 ±8 *	126 ±6	134 ±4	127 ±6	121 ±5
Heart Rate (/min)	155 ±14	184 ±9 *	171 ±8	171 ±10	178 ±8	165 ±8	148 ±12	170 ±7	174 ±14
Blood Glucose (mg/dl)	124 ±2	119 ±3	123 ±2	119 ±3 *	117 ±3	120 ±1	121 ±2	118 ±2	121 ±1

*paired comparison to 0 time = $p < 0.05$

FIGURE LEGENDS

Figure 1. Mean systemic arterial pressure and heart rate changes
(Mean ± SE) in perfused and nonperfused dogs infused with endotoxin from
0 to 30 minutes. Groups A, C, and D. (Number of survivors/total number
dogs studied).

�✱= $p < 0.05$, paired comparison with 0 time

■= $p < 0.05$, paired comparison with -90 minutes

↓= cessation of perfusion

Figure 2. Changes in blood glucose concentrations and whole blood
clotting times (Mean ± SE) in perfused and nonperfused dogs infused with
endotoxin from 0 to 30 minutes. Groups A, C, and D (Number of survivors/
total number dogs studied).

✱= $p < 0.05$, paired comparison with 0 time

■= $p < 0.05$, paired comparison with -90 minutes

▲= $p < 0.05$, unpaired comparison of Group D with Groups A and C

↓= cessation of perfusion

Figure 3. Changes in circulating white blood cell and platelet concen-
trations (Mean ± SE) in preperfused, perfused and nonperfused dogs infused with
endotoxin from 0 to 30 minutes. Groups A, C, and D. (Number of survivors/
total number dogs studied).

✱= $p < 0.05$, paired comparison with 0 time

■= $p < 0.05$, paired comparison with -90 minutes

▲= $p < 0.05$, unpaired comparison of Group C with Group A

↓= cessation of perfusion ·

Figure 4. Mean systemic arterial pressure responses (Mean ± SE) of
dogs with and without preperfusion and with and without exogenously administered
heparin to infusion of endotoxin from 0 to 30 minutes. Group A compared with

E. Group C compared with F. (Number of survivors/total number dogs studied)

✶ = p < 0.05, paired comparison with 0 time

■ = p < 0.05, paired comparison with -90 minutes

▲ = p < 0.05, unpaired comparison of Group C with F

↓ = cessation of perfusion

Figure 5. Changes in white blood cell and platelet concentrations
(Mean ± SE) in dogs nonperfused or preperfused with or without heparin given
endotoxin 0 to 30 minutes. Groups A, C, and F. (Number of survivors/total
number dogs studied).

✶ = p < 0.05, paired comparison with 0 time

■ = p < 0.05, paired comparison with -90 minutes

↓ = cessation of perfusion

Figure 6. Mean changes in mean systemic arterial pressure, whole blood
clotting time, platelets, fibrinogen, and factor V in intact dogs given endo-
toxin and dogs preperfused with and without heparin given endotoxin. (Endotoxin
infused from 0 to 30 minutes). Groups A, C, and F. (Number of survivors/
total number dogs studied).

✶ = p < 0.05, paired comparison with 0 time

■ = p < 0.05, paired comparison with -90 minutes

▲ = p < 0.05, unpaired comparison between groups

↓ = cessation of perfusion

Figure 1

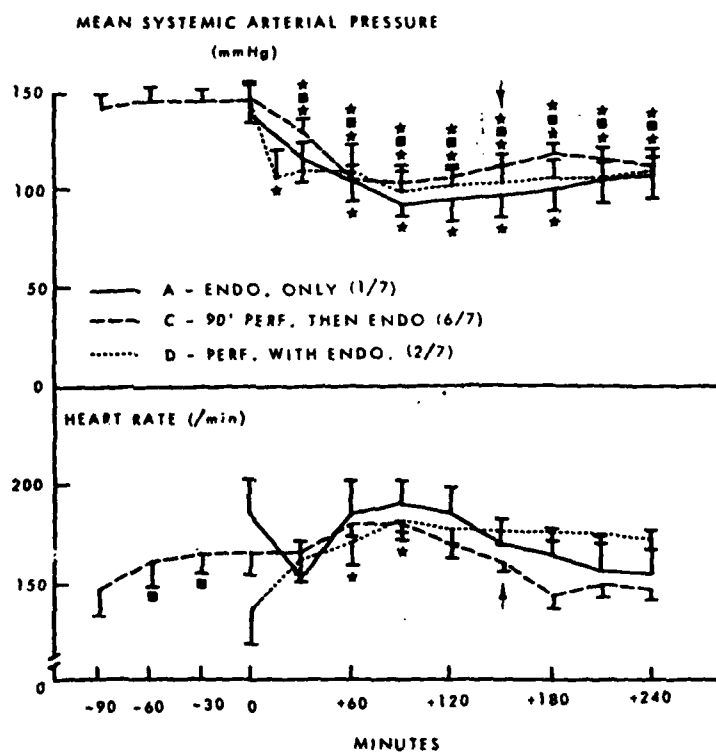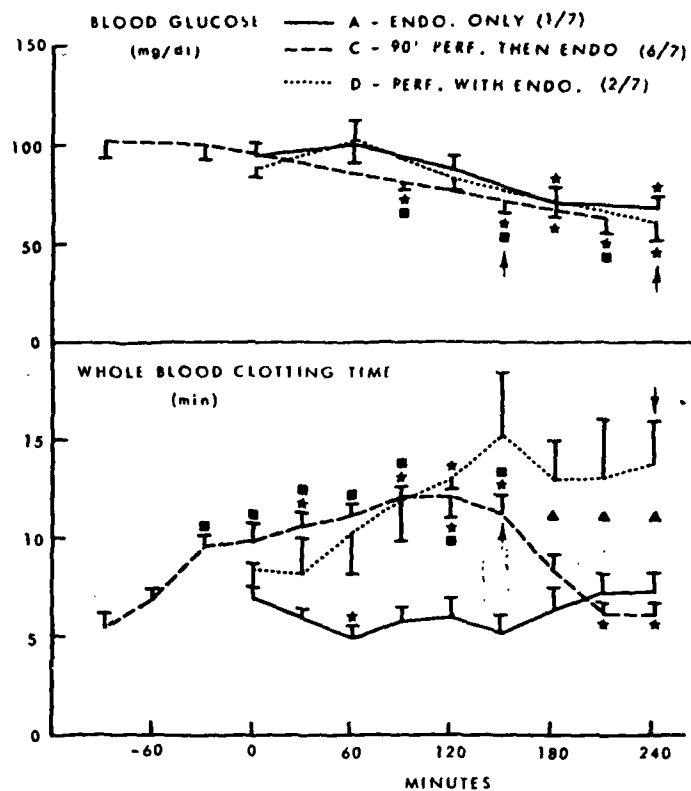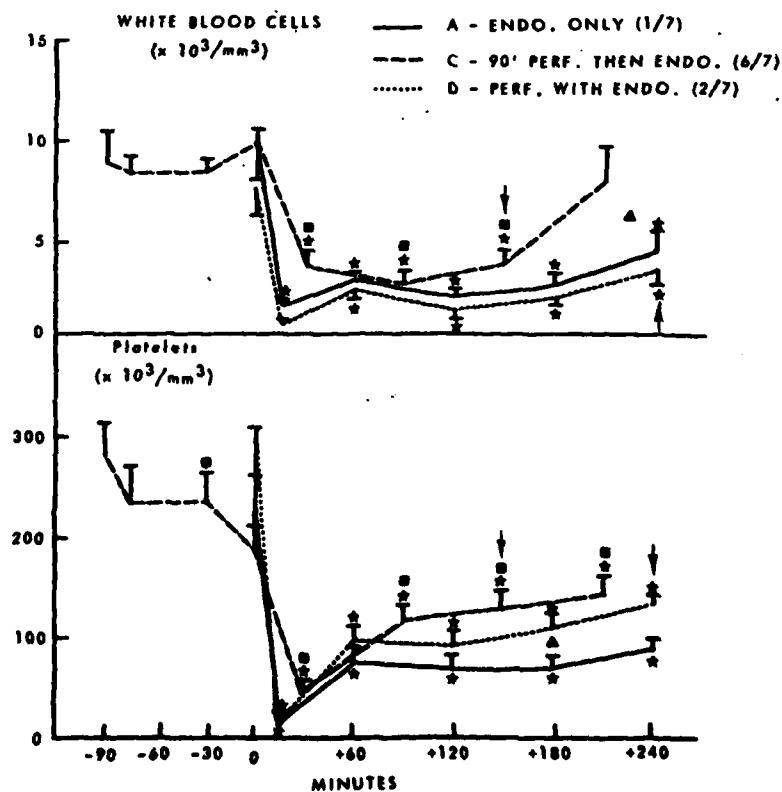
MEAN SYSTEMIC ARTERIAL PRESSURE
(mmHg)

A - ENDO. ONLY (1/7)
C - 90' PERF. THEN ENDO (6/7)
D - PERF. WITH ENDO. (2/7)

HEART RATE (/min)

-90 -60 -30 0   +60   +120   +180   +240

MINUTES

Figure 2

Figure 3

Figure 4
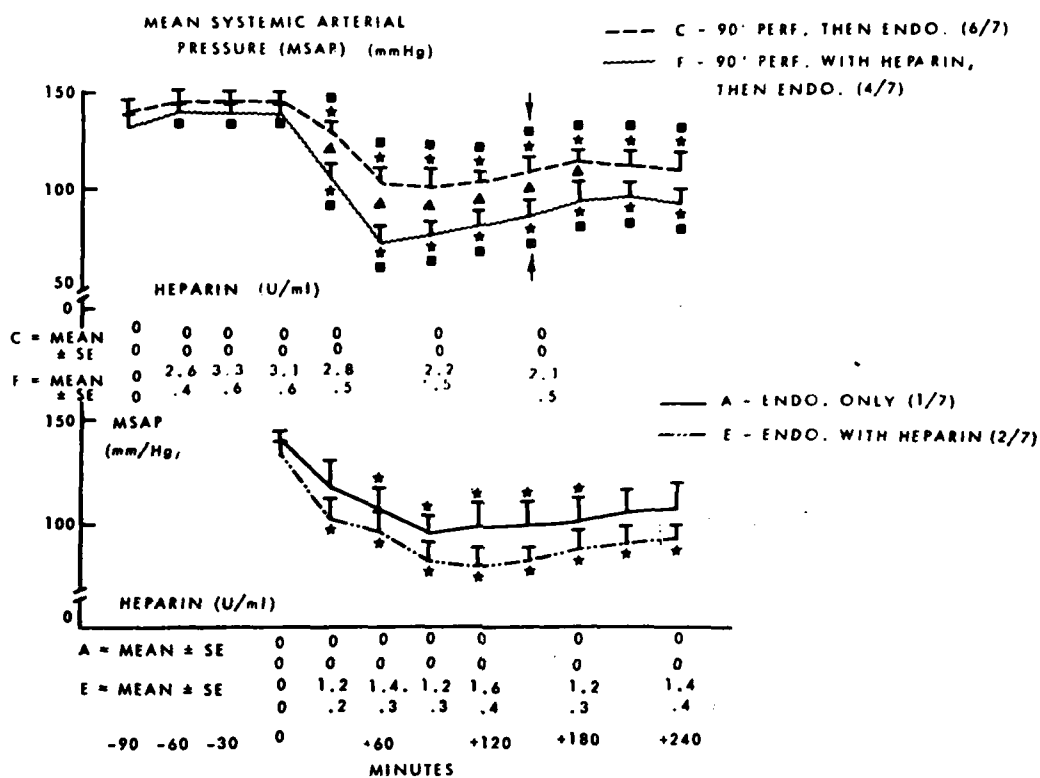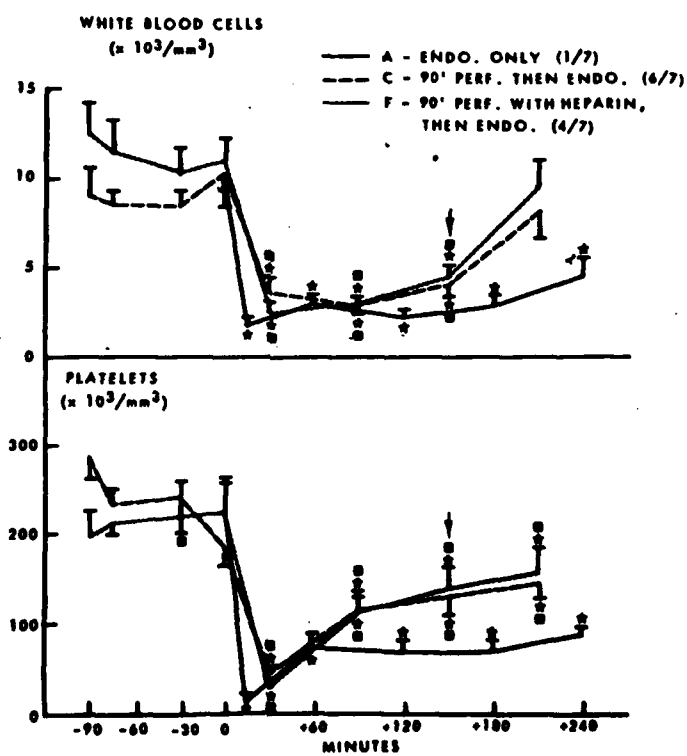
MEAN SYSTEMIC ARTERIAL PRESSURE (MSAP) (mmHg)

- - - C - 90' PERF. THEN ENDO. (6/7)
—— F - 90' PERF. WITH HEPARIN, THEN ENDO. (4/7)

HEPARIN (U/ml)

C = MEAN ± SE	0 0	0 0	0 0	0 0	0 0	0 0	0 0	
F = MEAN ± SE	0 0	2.6 .4	3.3 .6	3.1 .6	2.8 .5	2.2 .5	2.1 .5	

—— A - ENDO. ONLY (1/7)
—·—·— E - ENDO. WITH HEPARIN (2/7)

MSAP (mm/Hg)

HEPARIN (U/ml)

A = MEAN ± SE	0 0	0 0	0 0	0 0	0 0	0 0	0 0	
E = MEAN ± SE	0 0	1.2 .2	1.4 .3	1.2 .3	1.6 .4	1.2 .3	1.4 .4	

-90 -60 -30 0 +60 +120 +180 +240
MINUTES

Figure 5

WHITE BLOOD CELLS (× 10³/mm³)

—— A - ENDO. ONLY (1/7)
- - - C - 90' PERF. THEN ENDO. (6/7)
—— F - 90' PERF. WITH HEPARIN, THEN ENDO. (4/7)

PLATELETS (× 10³/mm³)

-90 -60 -30 0 +60 +120 +180 +240
MINUTES

Figure 6
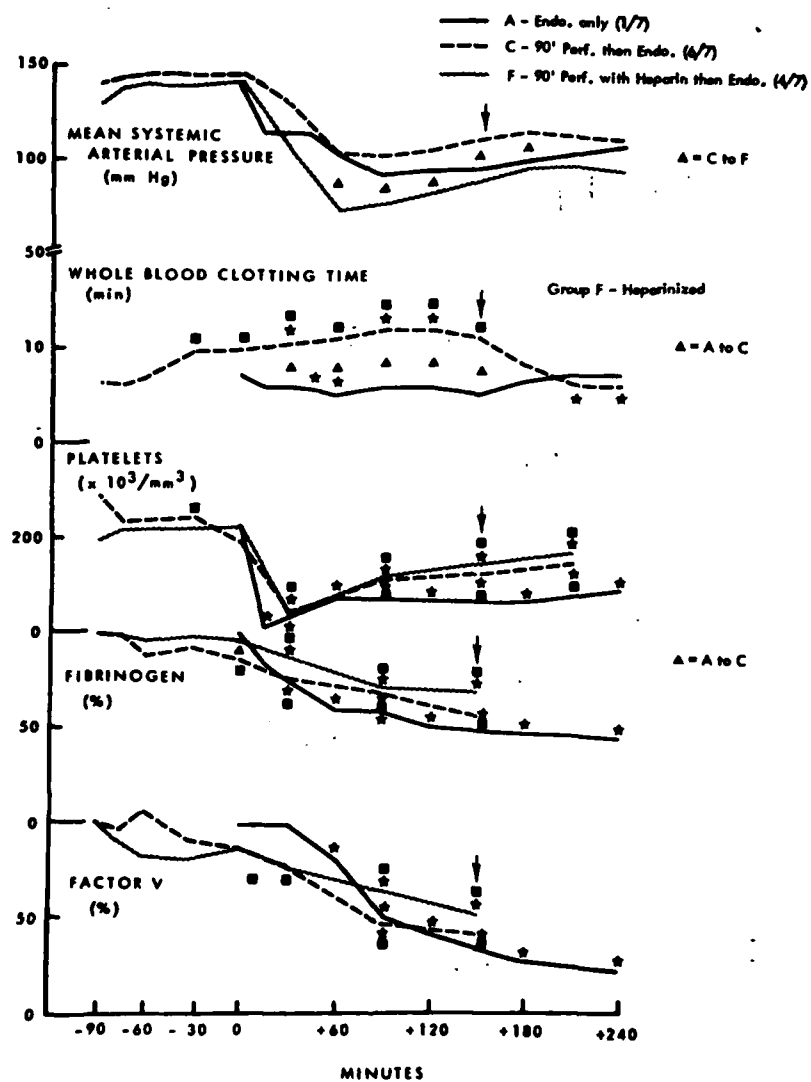
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white blood cell changes in shock, survival from endotoxin shock, endotoxin shock, therapy of shock, perfusion during shock, coagulation changes in endotoxin shock

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*
Our previous work demonstrated that after 90 minutes of arteriovenous perfusion without anticoagulation dogs were protected from lethal endotoxin. The present study extended that work by conducting experiments with hearpinized and nonheparinized, perfused and nonperfused animals administered endotoxin. Experiments with 42 adult mongrel dogs were divided into six groups: Group A (endotoxin; no perfusion); B (perfusion; no endotoxin); C (perfusion endotoxin initiated after 90 minutes); D (perfusion and endotoxin simultaneously); E (endotoxin plus heparin; no perfusion); F (perfusion plus heparin; endotoxin

initiated after 90 minutes). Percent permanent survival (>7 day): Group A= 14%; Group B= 100%; Group C= 86%; Group D= 29%; Group E= 29%; Group F= 57%. Survival results from Group A document the degree of lethality of the endo- toxin. Observations from Group B confirm the physiological stability of the perfusion preparation. Findings from Group C document that 90 minutes pre- perfusion provides excellent defense against the lethality of endotoxin shock. Whole blood clotting time (WBCT) decreased in nonperfused dogs (Group A), following endotoxin while perfusion alone (Group B) and perfusion followed by endotoxin (Group C) were associated with increases in WBCT and numbers of immature neutrophils. Arterial pressure and blood glucose, total white blood cell, platelet, fibrinogen, and factor V concentrations were unaffected by perfusion but fell sharply in all groups after endotoxin. The decrease in numbers of neutrophils following endotoxin was much less, and the increase in immature neutrophils was significantly greater, in the preperfused animals which had received endotoxin (Group C) than in the animals that received endo- toxin alone (Group A). Results underscore the following: (a) Arteriovenous extracorporeal pump perfusion without exogenous anticoagulation is not harm- ful: All animals were permanent (>7 day) survivors. (b) Death from endotoxin is prevented by preperfusion of 90 minutes but not when endotoxin is adminis- tered at the onset of perfusion. (c) Intravenous heparin administration offers no discernible protection against endotoxin shock in either perfused or non- perfused animals. (d) Increased numbers of circulating immature neutrophils are associated with protection from the lethal effects of endotoxin. This may be an important marker of the adequacy of host response to endotoxin. (e) Increasing venous return to the heart by an arteriovenous perfusion system is not a significant mechanism responsible for protecting animals administered endotoxin.

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